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Introduction to the CEA family: *structure, function and secretion*

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ABSTRACT: Due to the phenomenal progress in the field of tumor immunology that took place during the last twenty years, we dispose today of highly specific and sensitive techniques and reagents like monoclonal antibodies (MAbs). In this context the discovery in human carcinomas of tumor-associated antigens, such as CEA, was of primary importance, especially since the latter was found to have clinical relevance as a tumor marker. Based on animal models, a new in vivo *technology for the detection of tumors and metastases was developed in recent years, that uses anti-CEA MAbs, or fragments of them, coupled to radio-isotopes. This technique, called radio-immunodetection (RAID), also paved the way for immunotherapeutic procedures, where again CEA served as the target-antigen. This new technique holds great promise, provided the epitope-specificity of the MAbs is well-controlled: it has been shown that CEA belongs to a large gene-family of at least 22 members, which can be subdivided into two subgroups (i.e., the CEA- and the PSG-subgroup) and which in turn belongs to the immunoglobulin-supergene family. Great structural similarities render the distinction of the various cross-reactive molecules by immunological means rather difficult. (Int J Biol Markers, 1992; 7: 132-6)*

KEY WORDS: LEA gene, Structure, Molecular biology

At present the exact characterization of tumor antigens is one of the most important and most active fields of experimental tumor immunology. It is virtually the basis of all investigations concerning the possible role such substances may play in immune surveillance, immune recognition and defense mechanisms of malignant cells in clinical oncology, in immunodiagnosis, and immunotherapy.

Since results that have been obtained with virus- or chemically-induced mouse tumors are not even valid for all strains of mice and much less for human tumors, the discovery of the carcinoembryonic antigen (CEA) in man cannot be overemphasized.

Indeed, little more than 25 years ago two groups, Gold and Freedman in Canada and von Kleist and Burtin in France, described independently the presence of this tumor antigen in colonic carcinomas and their metastases (1, 2). It was called carcinoembryonic antigen because it was also shown to be present in the homologous embryonic tissues. The name is *strictu senso* incorrect, because CEA is produced not only during the first trimester of gestation, but throughout pre-natal life and even after birth. When it was shown that CEA circulated in the blood of cancer patients, the hardly characterized antigen was immediately tried for its usefulness in clinical oncology, which was rapidly proven. So this antigen became world-wide known as the first truly useful tumor marker, before anyone had the time or possibility to study and define its complicated molecular nature.

Although Gold and Freedman in their first paper in 1965 already described the presence of carcinoembryonic antigens (1), they were quoted thereafter of having discovered a CEA, and it took almost a decade before it was generally accepted that CEA was not an entity, but rather a member of a heterogeneous family of more or less closely related cross-reactive glycoproteins, which could not be clearly distinguished by the technical means then currently employed, for instance the double-immunodiffusion or immunoelectrophoresis with monospecific antisera or the characterization of physico-chemical properties.

Table I summarizes the properties thought essential at that time for defining the CEA, and any human glycoprotein fulfilling these criteria was called CEA. When put in a test, a radioimmmuno-assay or enzymoimmuno assay, this CEA worked beautifully as a tumor marker in clinical hands and this, as everybody knows, till the present day. However, very soon it was obvious that these criteria were non-essential, because

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TABLE I - PHYSICO-CHEMICAL PROPERTIES OF CEA

- MW of 180,000 + 20,000 D
- beta electrophoretic mobility in neutral pH
- ip of 3.75 ± 0.25
- soluble in perchloric acid
- soluble in 50% saturated ammonium sulphate
- unsoluble in ethanol
- heat stable
- main amino acid: asparagine/aspartic acid
- main sugar: N-acetylglycosamine
- single polypeptide chain

they were shared by many CEA-related substances. In 1972 we described the first, most important of these cross-reactive antigens (3), which received the rather uncomitting name of non-specific cross-reacting antigen or NCA, a term which has now been generally accepted for this group of molecules, several of which have been described under various denominations, but were later found to be either identical to the NCAs or to belong to the CEA family as separate members.

Since the cross-reactions were first observed when monospecific but polyclonal antisera were employed, it was logic to explain them by crossreactions with CEA-like compounds and it was hoped to define specifically the CEA once these cross-reactions were eliminated by the development and use of CEA-reactive monoclonal antibodies. When this was not the case, it became quite evident that CEA not only was an almost ubiquitous, heterogeneous molecule, but also had very few specific and many shared epitopes.

It took another 15 years before the successful cloning of the genes and the characterization of cDNAs showed that the CEA-gene represents the prototype of a large gene-family, of which so far more than 30 members have been identified and there are indications that there are many more (4).

In 1989 the CEA gene-family has been officially reconstituted at an ISOBM-Workshop (5): according to consistent amino-acid sequence similarities it is divided into two subgroups: the CEA- and the PSG-subgroup, which are depicted in Table II.

The first subgroup comprises the genes encoding for CEA, which exists in a 180 kD and a 160 kD form and the genes of the other classical crossreacting antigens, like the NCA and the biliary glycoprotein BGPI, first described in human bile by Svenberg as a heavily glycosylated 85 kD protein, of which probably more than 10 different molecules exist (6). There are some further CEA family members (abbreviated by the prefix CGM), namely the CGM6, of which the gene product most probably is the NCA95, first described by

TABLE II - CLASSIFICATION OF THE HUMAN CEA GENE FAMILY

Old gene or clone name	New gene or mRNA name
1. CEA subgroup	
CEA	CEAa CEAb
NCA	NCA
hsCGM6, M6, NCA-W272	CGM6
BGPI, TM-1 CEA TM-2 CEA TM-3 CEA TM-4 CEA 4-22 4-13	BGPa BGPb BGPc BGPd BGPe BGPf
hsCGM1	CGM1
hsCGM2 5E8, 7E12	CGM2
2. PSG subgroup	
PSßG PSG93, PSßG-D, hPSP11, FL-NCA-2, hPS3, PSG1a PSG16 PSßG-C FL-NCA-1, PSGId, SG9 PSßG-Ci, PSG95 PSßG-D'	PSG1 PSG1a PSG1b PSGIc PSGId PSGIe PSG1f
PSßG-E, SG8	PSG2n
pSP1-i, hc17, PS35, hTS16, PSG A, SG5	PSG3m
PSG4, hsCGM4, hHSP2, PSG9 (PS_{kx})	PSG4 PSG4a
PSG5, PSßG HL, FL-NCA-3	PSG5 PSG5n PSG5m
hsCGM3, PSGGB PSG6 hPS12, PSG10	PSG6 PSG6r PSG6s
PSG7	PSG7
CGM35 hTS1	PSG8 PSG8a
PS34, hPS2, PSßG B	PSG11s PSG11w

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Buchegger et al. in 1984 and found in leukocytes and chronic myeloid leukemia (7), the CGM1, CGM2, and CGM7-9, for which the gene products have yet to be found. Another member of the CEA-family, a CEA cross-reacting antigen of 140 kD expressed on human lymphoid cell-lines, has only recently been discovered by Kuroki et al. (8). The size of the CEA gene-family as estimated from Southern analyses of total genome DNA indicated the existence of 10-13 genes; however, this number may change (4).

Only a few years ago it was shown that the pregnancy-specific glycoproteins (PSGs) genetically belong also to the CEA gene-family. This subgroup consists of at least 11, but most likely many more members, which according to the new nomenclature are numbered PSG1-PSG11. Several mRNA-products of PSG are differentially spliced (they are designated with a small letter, e.g. PSG1a, PSG1b etc.), which leads to many protein variants (9).

The pregnancy-specific glycoprotein 1 (PSG1) and SP-1, i.e. Schwangerschaftsprotein 1, were again described independently by two groups, i.e. Tatarinov and Masyukevich and Hans Bohn (10, 11). The PSGs are a group of very heterogeneous glycoproteins, which under physiological conditions are predominantly expressed by the placenta without, however, being exclusively specific for this tissue, since PSG has also been reported in testicular tissues, the salivary glands, and fetal liver (12). In malignant lesions PSGs are found mainly in trophoblastic tumors, however, in a small percentage of cases it has also been found in tissues of breast and colonic carcinomas and multiple myelomas as well as in a leukemia cell-line HL60 and in the sera of patients with various gynecological carcinomas (13).

The various members of one subgroup show a great similarity at the nucleotide level of about 89-95%, however, a significantly lower similarity of only 66-70% is seen between the two subgroups (14).

The molecular structure of the different members of the CEA gene-family up to a certain degree follows a similar pattern with 4 distinguishable regions:
1) All members of the gene-family contain a hydrophobic leader peptide (L) of 34 amino-acids, which is cleaved off and therefore missing in the mature protein.
2) It is followed by the N-terminal domain, which for the members of the CEA subgroup comprises 108 AAs, for the second subgroup PSG1-11 108-110 AAs. This characteristic domain for the CEA gene-family is void of cysteines, but was found nevertheless to resemble the variable region of immunoglobulins, for which reason the N-domain is also called IgV-like domain (15).
3) Following this domain the various members show a different number of repeats, for CEA A1-A3, B1-B3, of which only the first (A1, B1) is with 178 amino-acids alike for all members of the CEA subgroup. In contrast to the N-terminal domain, the repeated A- and B-domains contain cysteines, which in position and distance from each other resemble those of the constant (C) region of the heavy chains of the immunoglobulins. These regions are, therefore, called IgC-like domains (15).
4) Some members of the CEA-subgroup, of which the sequence is known and with the exception of BGP have a membrane-associated (M) carboxyterminal domain, which consists mostly of hydrophobic aminoacids, which are, however, cleaved off and are replaced by a glycosylphosphatidylinosytol (GPi), a so-called pick tail structure, which anchors the protein in the cell membrane (16).

BGP is a classical transmembrane protein with a carboxy-terminal region consisting of a transmembrane domain of 43 hydrophobic aminoacids, followed by a hydrophilic cytoplasmic region, which according to the different splice variants, is made up of a varying number of aminoacids (17).

All members of the PSG-subgroup have relatively short C-domains and this together with a lack of the M-domain, which is characteristic for these molecules, has probably also functional consequences in respect to the secretion. Most PSGs are easily secreted and it is as yet unknown whether there are PSG-variants, which, like CEA or NCA, are membrane-bound via a GPI-structure (18).

All genes of the two subgroups of the CEA gene-family are localized as clusters on the long arm of chromosome 19 at position q13.1-q13.3, possibly close to the genes coding for the TGF-ß1 and LIPE (19).

The finding, that in the CEA the A- and B-domains are combined to form 3 identical repeats, which with the N-domain were found to be very similar to the immunoglobulin variable and constant domains, respectively, suggested, that CEA and its related molecules belong to the Ig-supergene family (20), to which belong among others such well-known molecules as the T-cell receptor, the platelet-derived growth factor receptor, the MHC-class I and class II antigens, the N-CAM, etc. The functions of these various members have as common trait in that they are well-characterized and serve as cell-cell-interaction-, recognition-, or cell adhesion molecules. This for the first time gave a hint as to the possible function of CEA-family members and led to several *in-vitro* studies, which so far seem to show that CEA might,

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indeed, play a role in cell-cell-adhesion mechanisms (21). This will be discussed in the next paper.

In clinical oncology it is a well-known fact that CEA is present more often in tissues than in sera of tumor-bearing patients; the reason of this discrepancy is not known. By xenotransplantation experiments in nude mice we showed that it must be an intrinsic factor of the tumors, whether or not CEA is released into the circulation: there were CEA-positive tumors which did not release it in humans, but once transplanted secreted CEA immediately, and there were others that grew and produced CEA, but did not release it in the nude mouse (22). The mechanism of secretion is not yet known but actively studied. It may well be that a GPI-specific phospholipase D is involved: this enzyme is present in human sera and granulocytes. However, this has yet to be proved (23, 24).

Apparently it is still a long way to elucidate the function and role of CEA, which after all is an embryonic antigen first, before becoming a tumor antigen and its biological role should be compatible for the physiological as well as the pathological state.

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